Simultaneous Electrospray and Atmospheric Pressure Chemical Ionization: The Science Behind the Agilent Multimode Ion Source

Technical Overview

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Introduction

The invention and commercialization of electrospray ionization (ESI) was revolutionary when applied to the field of organic analytical chemistry. For the first time, chemically and thermally fragile molecules (proteins, drugs, environmental compounds, to name a few classes) could easily and reliably be analyzed in high sensitivity by LC/MS techniques. ESI is used today in thousands of laboratories around the world for routine high sensitivity analysis of these and other analytes. As a testimony to the utility of this technique, its inventor, Professor John Fenn, was one of the recipients of the Nobel Prize in chemistry in 2002.

As remarkable as the technique of ESI is, it does not ionize all organic compounds of interest that may be eluted from an HPLC. It has been estimated that about 80% of all analytes respond by ESI, leaving a significant fraction as undetectable. This limitation was noticed early in the development of ESI, and other ionization techniques were developed almost simultaneously to fill this need. The technique of atmospheric pressure chemical ionization (APCI) and the related atmospheric pressure photoionization (APPI) are the best-known alternatives.

Further study of ESI and APCI pointed up fundamental differences between them.[1–3] The spray conditions and voltage conditions for optimal ESI operation are very different than for optimal APCI operation. In optimized ESI, ion formation occurs in the following sequence:

1. A surface charge is induced on the emerging liquid by imposition of an external electrical field
2. The charged liquid is converted to a spray of droplets
3. The droplets shrink due to solvent evaporation, which increases the surface charge density
4. Droplets undergo fission as a consequence of the high surface field strength
5. When a droplet is small enough, ions are ejected from the droplet due to the high field strength at the surface of the droplet

Under optimal APCI conditions, ion formation occurs as follows:

1. A spray of droplets is formed
2. The spray is dried completely creating a mixture of solvent molecules and analyte molecules in the gas phase
3. The solvent molecules and analyte molecules pass through a corona discharge
4. The corona discharge ionizes the solvent molecules, creating solvent ions
5. The solvent ions transfer charge to the analyte molecules, creating analyte ions

From the above lists, it should be apparent that the spray formation and timing of ion generation are different in ESI and APCI. As a result of these differences, all MS manufacturers have developed dedicated optimized ion sources for each technique. Users who want to analyze a sample using both techniques generally must run the analysis using one source first, and if there is no response, run the sample on the instrument using the other source. This significantly decreases the number of samples per day that may be analyzed on a given instrument.

In response to this diminished throughput caused by the fundamental differences between ESI and APCI, novel ion sources have been developed to combine the two ionization techniques into one source.[4,5] The differences in performance of these combination (also known as "dual-mode" or "multimode") sources may be traced to how they address the incompatibilities between ESI and APCI. Do they perform the ionization in the same region of space, or do they separate the ionization regions and attempt to optimize the performance of each? And if the ionization regions are separate, how is the sample delivered to the two regions and how is the separation maintained? This document describes the features of the Agilent multimode ionization source and how it addresses these incompatibilities, permitting high-flow, high-sensitivity ESI and APCI to be performed independently or simultaneously on a single injection of a sample.

Experimental

All experiments were performed using an Agilent 1100 Series LC system comprised of a binary pump, autosampler, thermostatted column compartment; and an Agilent 1100 Series LC/MSD SL (G1956D) quadrupole mass spectrometer. The LC/MSD was equipped with a multimode source, dedicated ESI source, or dedicated APCI source as needed. The sources for the LC/MSD are all interchangeable. The multimode source is currently supported on G1946 LC/MSD instruments, models B, C, and D, and on all G1956 LC/MSD instruments, models A and B. However, some of these models need both hardware and software upgrades to be able to control the multimode source; see your Agilent representative for details.

The LC/MS conditions for the analysis of the samples in this note vary with sample type; therefore, they are stated in the sidebars in the appropriate section.

Results and Discussion

The Agilent multimode source is a compact ion source that fits on the left side of the LC/MSD quadrupole mass spectrometer. Some distinguishing features of the source are highlighted (Figure 1).

Concepts behind simultaneous (mixed mode) ESI and APCI operation

A functional view of this source is given in Figure 2. The top part of the source is the "ESI zone" where the conditions exist for optimal electrospray ion formation. The bottom part of the source contains the "APCI zone" where optimal conditions exist for this ionization process. Some of the functional elements of this source are also shown and are discussed in detail below.

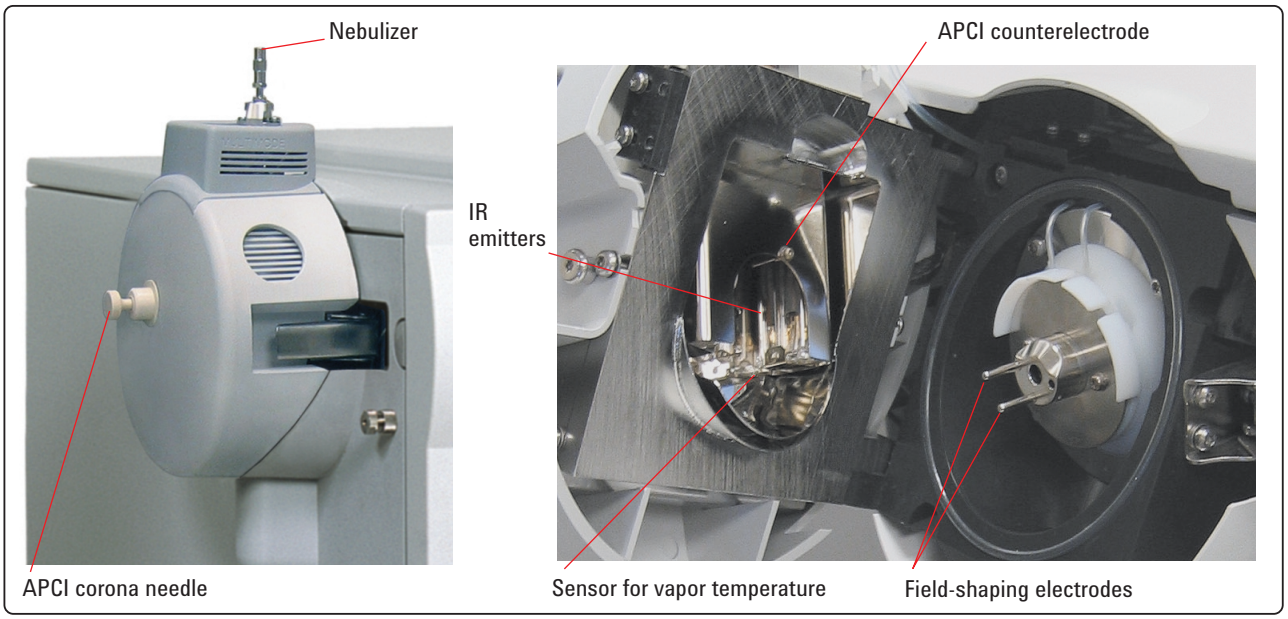

Figure 1. (left) The Agilent multimode ion source mounted on the MSD quadrupole mass spectrometer. (right) Source opened to show a few of the inner parts.

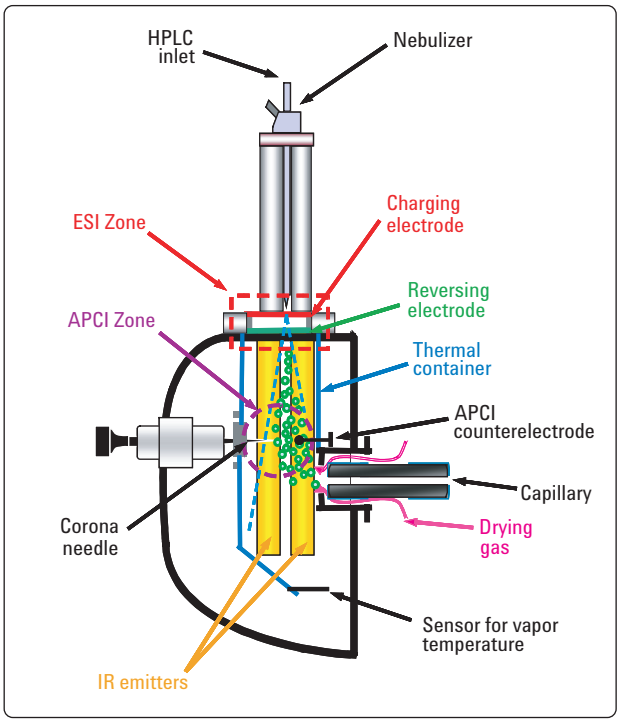

Figure 2. An overview diagram of the multimode source

ESI zone with nebulizer, charging electrode, and reversing electrode

This source uses the same patented orthogonal ESI geometry with a grounded nebulizer as is found on Agilent's dedicated ESI source. This design has proven to be extremely robust, directing the vast majority of solvent and spent droplets to waste while selectively diverting the ions to the capillary entrance of the mass spectrometer. The grounded nebulizer permits the mass spectrometer to be interfaced directly to other devices, e.g., capillary electrophoresis instruments, without fear of electrical interactions or special isolation precautions. The elements in the nebulizer are carefully optimized to provide the proper droplet size and size distribution for maximum production of ions. The charging electrode in the ESI zone (Figure 3) induces the surface charge on the liquid to start the ion formation, and ESI ions are generated here. The nebulizing gas flow pushes the droplets and ESI ions past the reversing electrode towards the APCI zone below.

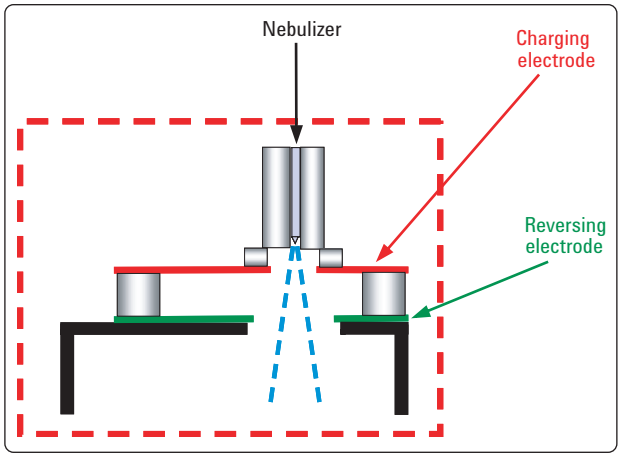

Figure 3. A close-up diagram of the ESI zone of the multimode source

Infrared emitters

A major innovation in this ion source is a set of infrared (IR) emitters (Figure 4) to aid in drying the aerosol. These emitters give off light in a broadband emission whose maximum is at 3 µm, the absorption wavelength of the hydrogen-bonded hydroxyl group of water. About 40% of the emitter's power occurs in this absorption band, and the emitter's maximum power output is 270 W, enough to completely and instantly vaporize 2 mL/min of 100% water and maintain the vapor temperature at 250 °C. For production of ESI ions, the emitters act in a similar role as heated drying gas, causing the droplets to shrink, undergo fission ("Coulombic explosion") and eject ions. Thus, the nitrogen drying gas consumption of an instrument using a multimode source is reduced considerably compared to that of an instrument with a dedicated ESI source using only heated drying gas. Typical nitrogen consumption is 5–7 L/min using the multimode source, whereas 12–14 L/min is usual for a standard ESI source. The power to the IR emitters is controlled by feedback from a vapor temperature sensor located in the exit gas flow stream of the multimode source. As the flow and/or mobile phase composition changes during an LC/MS analysis or during restoration of the initial gradient conditions, the emitter power changes to maintain the same vapor temperature (the "vaporizer" temperature as set in the software). This feedback control maintains ESI and APCI at the conditions for optimal performance and minimizes decomposition of thermally sensitive analytes that may be present.

APCI zone with IR emitters, corona needle, and APCI counter-electrode

The droplets continue down into the thermal container that surrounds the APCI zone of the source. Here, radiation from the IR emitters completely converts the droplets to solvent and analyte vapor to create the conditions for optimal APCI. A corona discharge is struck between the APCI needle and a counter-electrode (Figure 5). This discharge ionizes the solvent molecules which subsequently transfer their charges to the analyte molecules,

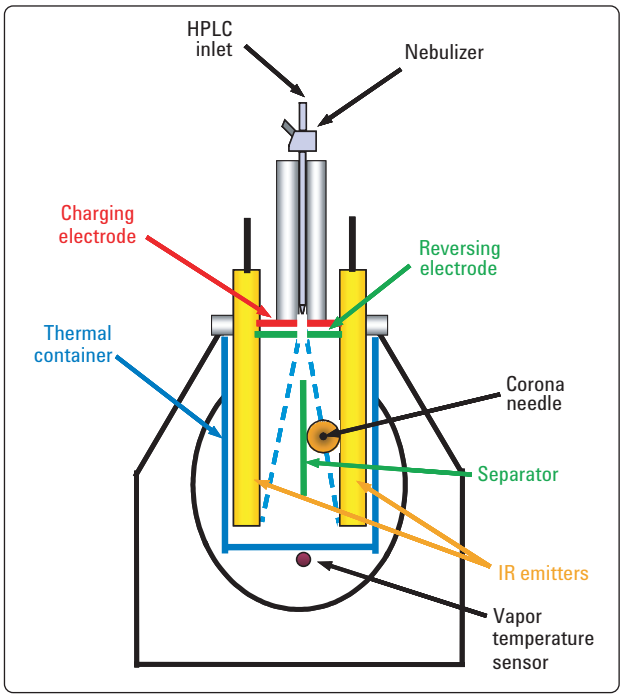

Figure 4. Diagram of the multimode source showing the relationship of the IR emitters and vapor temperature sensor with respect to other parts of the source

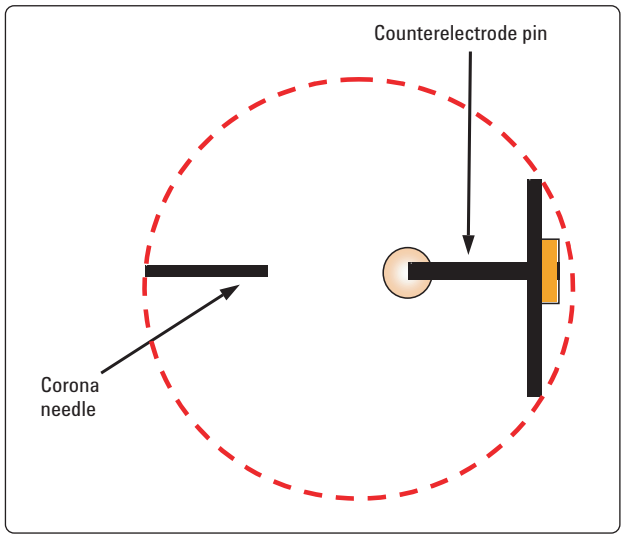

Figure 5. A close-up diagram of the APCI zone of the multimode source

producing ions. A separator in the middle of the source screens most of the ESI ions from reaction with the corona discharge present in the APCI zone. The ESI ion stream on one side of the separator and the APCI ion stream from the other side combine below the separator and are guided simultaneously into the capillary entrance of the MSD.

Simultaneous (mixed mode) ESI and APCI

From the above discussion, it may be seen that the design of the multimode source solves the problems of maintaining separate (but connected) ESI and APCI regions in a simple yet elegant fashion. The liquid from a single nebulizer is first charged and converted to an aerosol in the presence of an electrical field, the conditions for optimal ESI ion formation. Then, the residual droplets and ESI ions are pushed past the reversing electrode into the heated region on their way to the APCI zone.

The reversing electrode is the electrical boundary between the ESI and APCI zones, and without it ions would tend to migrate back toward the charging electrode instead of forward toward the mass spectrometer entrance. In the thermal container space between the ESI and APCI zones, the IR emitters convert the residual droplets to vapor while having no effect on any ESI ions that may be present. Once the vapor reaches the APCI zone, the conditions exist for optimal APCI ion formation. Thus, simultaneous efficient production of ESI and APCI ions is a function of the overall design of the source and the interaction of its unique parts.

If desired, the multimode source also functions well in ESI-only or APCI-only modes. To perform only ESI, the APCI corona needle voltage is turned off while leaving the ESI charging electrode voltage on, and only ESI ions are produced. To perform only APCI, the ESI charging electrode is turned off while leaving the APCI corona needle voltage on, and only APCI ions are produced. These voltages are under data system control, and thus the instrument may be switched between ESI, APCI, and mixed mode operation manually with a click of the mouse, automatically during an analysis, or automatically during a sequence.

Proof of concept: ESI-only, APCI-only and simultaneous (mixed mode) ESI and APCI

The proof of concept for simultaneous ESI and APCI operation is shown in Figure 6. A mixture of bovine insulin and indole was infused into the multimode source mounted on the LC/MSD quadrupole system. Bovine insulin, like all proteins possessing multiple basic sites, yields a characteristic multiple-ion spectrum in positive ESI mode; it has no response in APCI mode. Indole, being a small molecule with a weakly basic site, protonates poorly under ESI conditions but responds well in positive APCI mode. When a mixture of the two was analyzed using the multimode source, bovine insulin had a strong response in ESI-only mode, whereas indole had a very weak response. The opposite was true in APCI-only mode, with indole having a strong response. Both compounds responded well when the simultaneous ionization mode was enabled. This and other tests indicate that the multimode source produces ESI and APCI spectra similar to those of the dedicated sources and that the two ionization modes may be switched off, on, or combined for simultaneous ionization.

LC Conditions

Column:	None (infusion)
	0.1% acetic acid
Flow rate:	0.1 mL/min

MS Conditions

Source:	Multimode, positive ESI-only, APCI-only, or mixed mode
Drying gas flow:	5 L/min
Nebulizer:	40 psig
Drying gas temp:	350 °C
Vaporizer temp:	200 °C
Capillary voltage:	2500 V
Corona current:	2 µA
Peakwidth:	0.2 min
Time filter:	On
Scan:	100–3000 *m/z*
Fragmentor:	150 V
Stepsize:	0.1 *m/z*

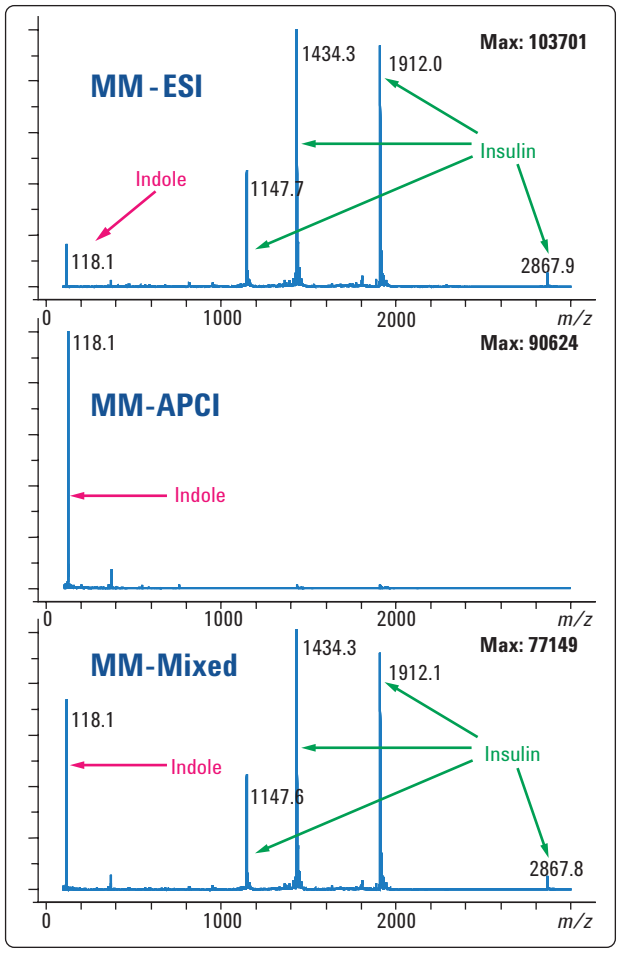

Figure 6. Proof of concept of simultaneous (mixed mode) ESI and APCI using a mixture of indole (50 pmol/µL) and bovine insulin (9 pmol/µL). (top) Source in ESI-only mode, exhibiting a response from insulin with a weak response from indole. (middle) Source in APCI-only mode, with only indole showing a response. (bottom) Source in mixed mode, with both indole and insulin showing strong responses.

Another example of this performance is shown in Figure 7. This was a mixture of four compounds, each of which responded most strongly in a single one of four possible modes: negative ESI, negative APCI, positive ESI, or positive APCI. By setting the ionization parameters to one of these possibilities, e.g., positive ESI mode, only the response of crystal violet was seen. However, injection of the same mixture using mixed mode operation and positive/negative polarity switching allowed detection of all four compounds using a single injection and analysis. A broad range of response like this is a welcome feature for analyses such as compound screening and high throughput analysis in early drug discovery.

LC Conditions

Column:	30 x 2.1 mm ZORBAX SB-C18, 3.5 µm (Agilent part no. 873700-902)
Mobile phase:	35:65 water:methanol with 0.2% acetic acid
Gradient:	None (isocratic)
Flow rate:	0.4 mL/min
Column temp:	Room
Injection volume:	1 µL

MS Conditions

Source:	Multimode, mixed mode with positive/negative switching
Drying gas flow:	5 L/min
Nebulizer:	60 psig
Drying gas temp:	350 °C
Vaporizer temp:	200 °C
Capillary voltage:	1000 V (positive and negative)
Corona current:	2 µA
Peakwidth:	0.2 min
Time filter:	On
SIM ions	
(positive mode):	372.2 m/z (crystal violet), 168.1 m/z (carbazole)
(negative mode):	165.1 m/z (hexanesulfonic acid), 193.1 *m/z* (9-phenanthrol)
Fragmentor:	
(positive mode):	160 V at 168.1 *m/z*, 180 V at 372.2 *m/z*
(negative mode):	140 V at 165.1 *m/z*, 160 V at 193.1 *m/z*

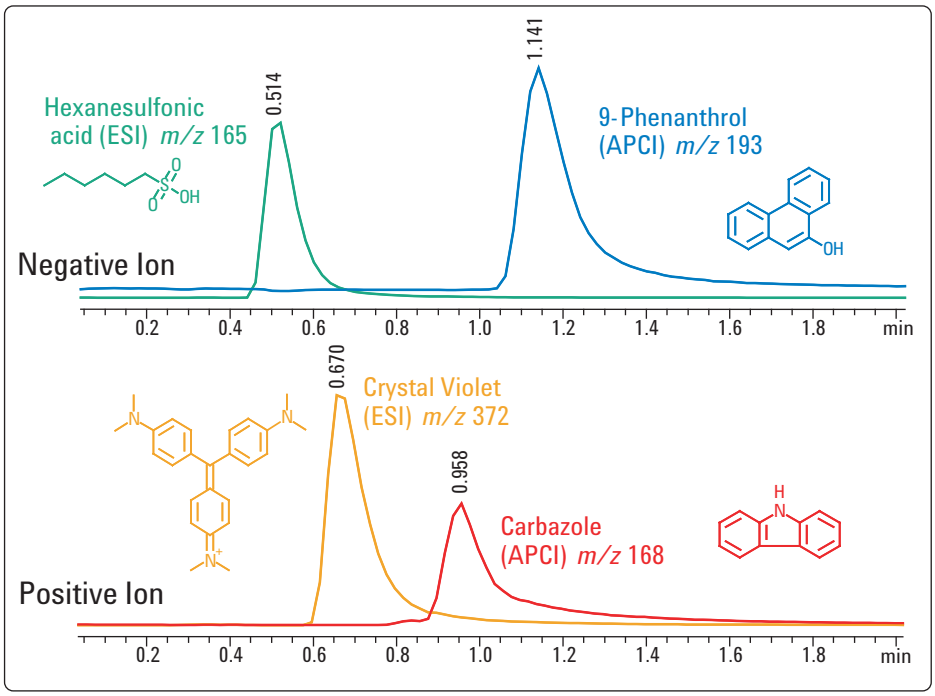

Figure 7. Proof of concept of mixed mode operation with positive/negative polarity switching using the multimode source demo sample. These four compounds each respond primarily in only one ionization mode and only one polarity but may be detected in a single analysis.

Infrared emitters provide conditions for optimal APCI

APCI requires different spray and voltage conditions than ESI for maximum production of ions. It has been known for many years that the best APCI performance is obtained when both the solvent and the analyte are in the vapor state. All instrument manufacturers, including Agilent, have additional heating on their dedicated APCI sources or APCI probes to achieve these conditions. The infrared emitters of the multimode source accomplish the same feat, completely vaporizing the HPLC effluent and any analytes it contains, to produce this vapor. Figure 8 shows replicate injections of diphenhydramine, a compound that responds well by ESI and APCI, at an HPLC flow rate of 0.4 mL/min. As the vaporizer temperature was increased from 60 °C to 250 °C, the HPLC effluent was converted essentially completely to the vapor state, and the APCI response increased approximately five-fold.

LC Conditions

Column:	30 x 2.1 mm ZORBAX SB-C18, 3.5 µm (Agilent part no. 873700-902)
Mobile phase:	50:50 water:acetonitrile
Gradient:	None (isocratic)
Flow rate:	0.4 mL/min
Column temperature:	Room
Injection volume:	1 µL

MS Conditions

Source:	Multimode, positive APCI-only
Drying gas flow:	5 L/min
Nebulizer:	20 psig
Drying gas temp:	350 °C
Vaporizer temp:	60, 115, 250 °C
Capillary voltage:	2500 V
Corona current:	6 µA
Peakwidth:	0.05 min
Time filter:	On
SIM ions:	167.1, 256.2 *m/z*
Fragmentor:	120 V

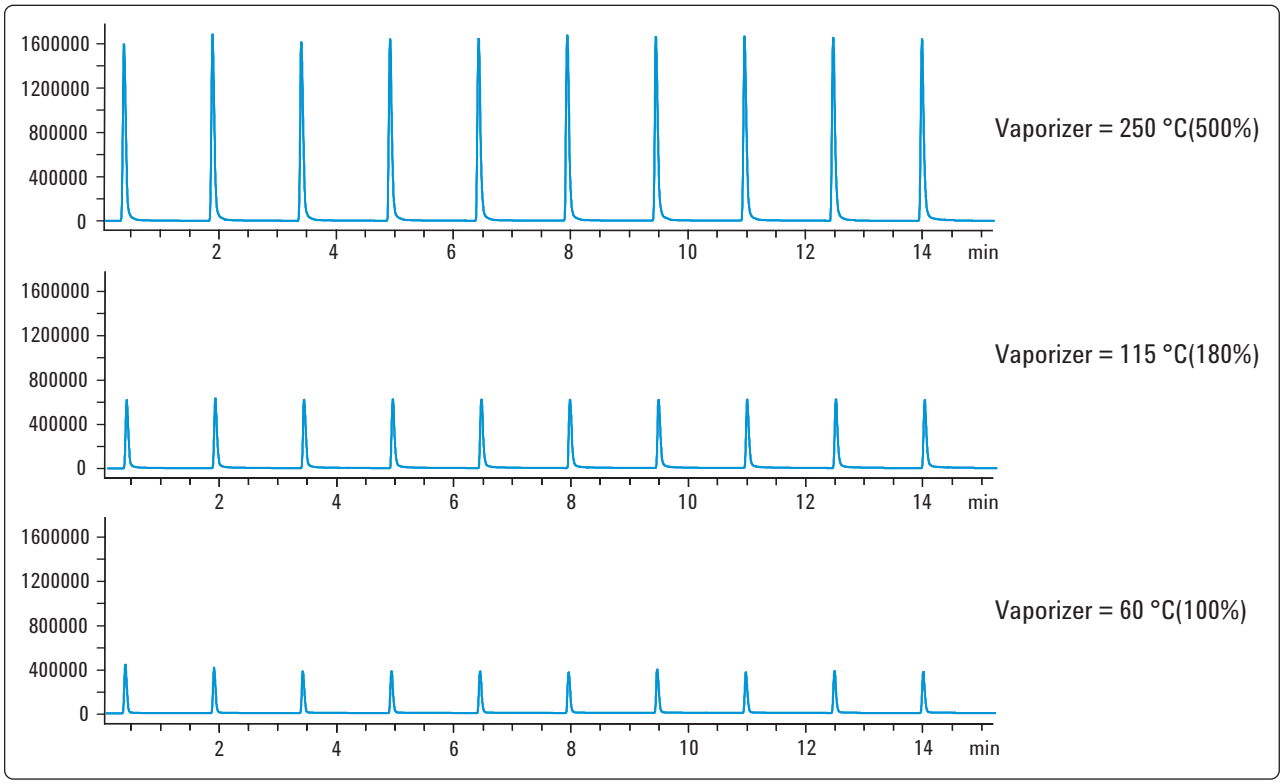

Figure 8. Improvement in diphenhydramine response with increasing IR emitter vaporizer temperature. Response increased by 5X over the temperature range of the experiment.

A more dramatic illustration of this effect is shown in Figure 9. The compound here is reserpine, also a compound that responds well by both ESI and APCI and is generally used to test the sensitivity of LC/MS systems. Reserpine has a lower vapor pressure than diphenhydramine when compared at the same temperature, so greater vaporizer temperatures are needed to convert the analyte into the gas phase. The experiment consisted of performing five consecutive injections of reserpine, 10 pg each, increasing the vaporizer temperature by 25 °C, performing another five injections, and so forth up to the temperature limit. Then the vaporizer was allowed to cool to its starting temperature while injections of reserpine were continued. The flow rate and mobile phase during this experiment was 1.0 mL/min of 75:25 methanol:water with 5 mM ammonium formate, typical for APCI, which performs better at higher flow rates.

The signal response for reserpine increased almost 17 times as the vaporizer temperature was increased. Furthermore, the response of the emitters was quite rapid. The command to raise the temperature occurred simultaneously with an injection. In the 20 seconds that elapsed while the analyte traversed the tubing and column from the ALS to the ion source, the gas temperature essentially reached and stabilized at the new value, 25 °C higher. The source also cooled reasonably rapidly, dropping from 250 °C to 125 °C in about three minutes. A final point of this figure is that the increased signal also resulted in better reproducibility of the measured response. Data obtained at the lowest vaporizer temperature, 125 °C, had a higher %RSD value of 13% (four replicates) compared to the data taken at higher temperatures.

LC Conditions

Column:	30 x 2.1 mm ZORBAX SB-C18, 3.5 µm (Agilent part no. 873700-902)
Mobile phase:	25:75 water:methanol with 5 mM ammonium formate
Gradient:	None (isocratic)
Flow rate:	1.0 mL/min
Column temperature:	Room
Injection volume:	1 µL, 5 injections at each vaporizer temperature

MS Conditions

Source:	Multimode, APCI-only
Drying gas flow:	5 L/min
Nebulizer:	20 psig
Drying gas temp:	350 °C
Vaporizer temp:	125—250 °C in 25 °C steps
Capillary voltage:	–3000 V
Corona current:	6 µA
Peakwidth:	0.1 min
Time filter:	On
SIM ion:	609.3 *m/z*
Fragmentor:	200 V

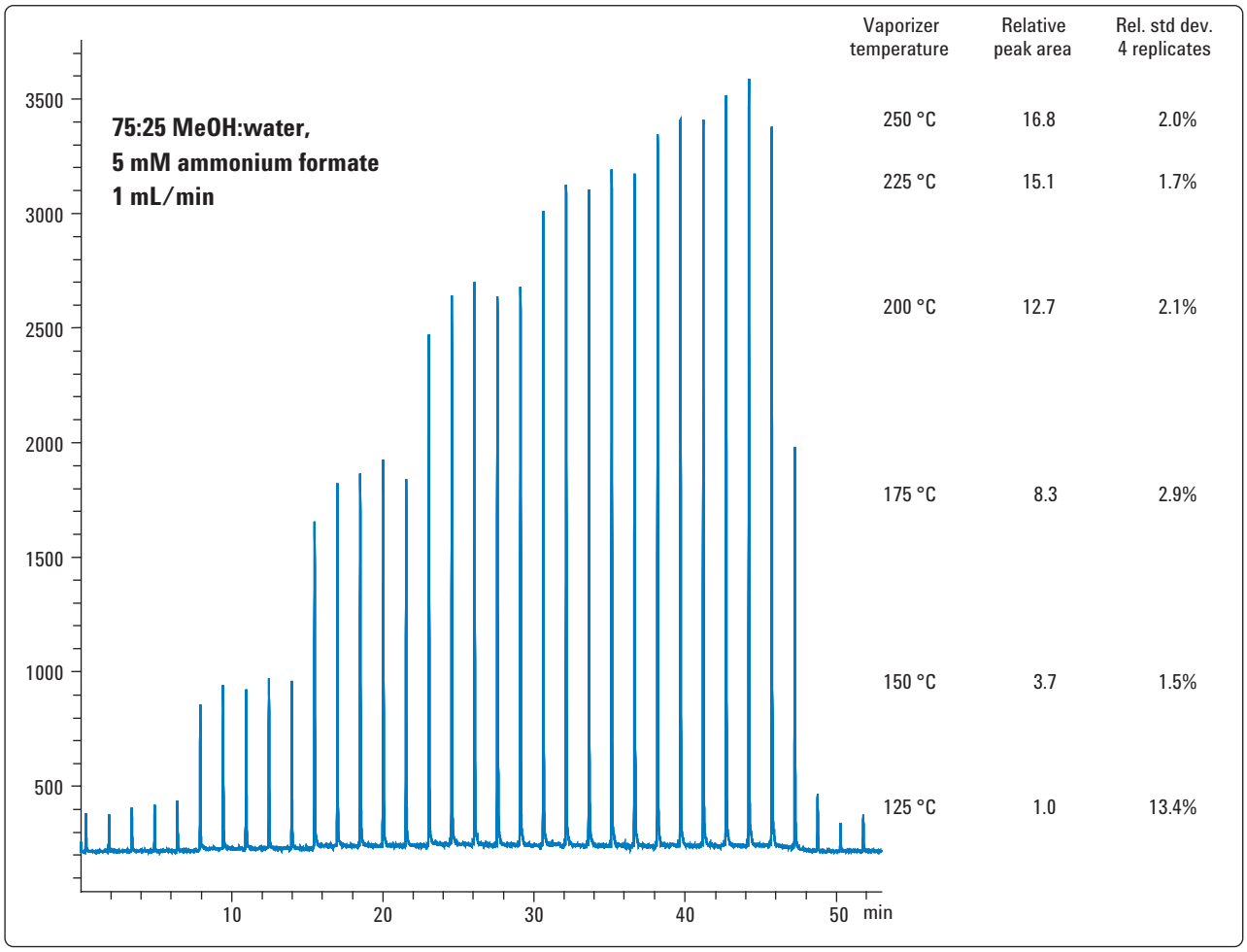

Figure 9. Improvement in response of repetitive 10 pg reserpine injections with increasing IR emitter vaporizer temperature. Response increased by 16.8X over the temperature range of the experiment. The data also shows the rapid controlled response of the IR emitters to a 25 °C step change in the setpoint.

Good sensitivity in ESI, APCI, and mixed mode

The multimode source can achieve detection limits in the low picogram range in all modes of operation. Shown in Figure 10 are replicate injections of the compound reserpine. As is noted on the left side of the figure, the measured sensitivity was 36 in positive APCI mode (top), 32 in positive ESI mode (middle), and 36 in positive mixed mode (bottom). The source produced comparable signal/noise values for this compound in all modes, suggesting that mixed mode operation achieved essentially the same sensitivity as obtained in a dedicated mode. Furthermore, these sensitivity values were similar to a dedicated ESI or dedicated APCI ion source on the same instrument (data not shown; current sensitivity specifications for this instrument are 20:1 signal/noise with either a dedicated ESI or dedicated APCI source). There is typically no compromise in sensitivity for the analysis of a single compound analyzed under the same conditions.

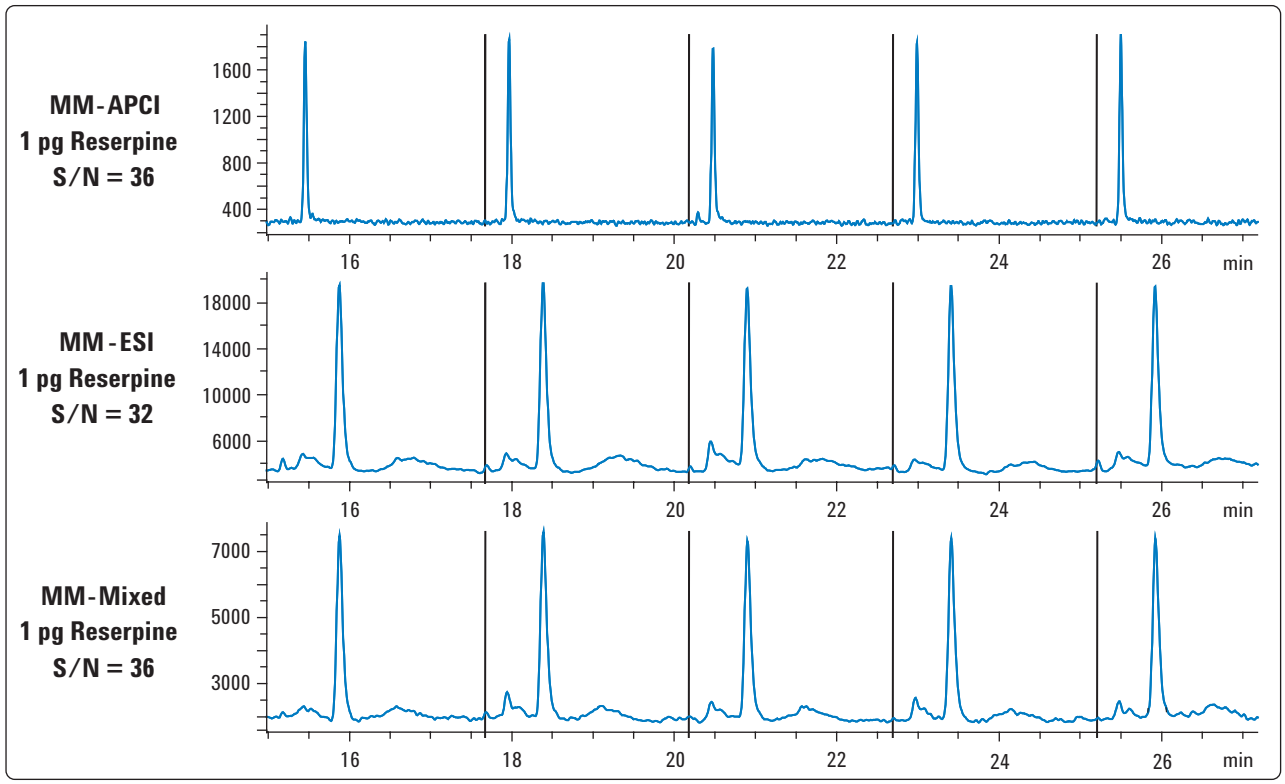

Figure 10. Typical sensitivity data for the multimode source in positive polarity operation, repetitive 1 pg reserpine injections. (top) APCI-only mode, (middle) ESI-only mode, (bottom) mixed mode operation.

LC Conditions

Column:	30 x 2.1 mm ZORBAX SB-C18, 3.5 µm (Agilent part no. 873700-902)
Mobile phase:	25:75 water:methanol with 5 mM ammonium formate
Gradient:	None (isocratic)
Flow rate:	0.4 mL/min ESI and mixed mode, 1.0 mL/min APCI
Column temperature:	Room
Injection volume:	1 µL

MS Conditions

Source:	Multimode, positive APCI-only, ESI-only, or mixed mode
Drying gas flow:	5 L/min
Nebulizer:	20 psig APCI, 60 psig ESI, 40 psig mixed-mode
Drying gas temp:	350 °C
Vaporizer temp:	250 °C
Capillary voltage:	3000 APCI, 2000 V ESI and mixed-mode
Corona current:	6 µA APCI, 2 µA mixed mode
Peakwidth:	0.08 min ESI and mixed-mode, 0.04 min APCI
Time filter:	On
SIM ion:	609.3 *m/z*
Fragmentor:	200 V

While the instrument with a multimode source has excellent response for a single compound, as shown above, this does not mean that all compounds in the same analysis will "respond well." ESI and APCI are chemical ionization processes. They often have different optimum source conditions for different analytes in a mixture. An example of this would happen if two compounds coeluted, but each needed a significantly different vaporizer temperature for optimal response. In this case, one must select a compromise temperature for the vaporizer. (Note that this situation is no different than exists for a dedicated ion source.) With the greater number of compounds that may be ionized with a multimode source in mixed mode, it is more likely that one will have to use a compromise setting for a parameter; however, the multimode source is designed to reduce the impact of those compromises.

Analysis of thermally labile compounds: IR emitters and temperature feedback

ESI is renowned for its ability to ionize thermally labile substances such as proteins and drugs, which in part gives rise to its great utility. As stated previously, the multimode source uses IR emitters to aid in drying the droplets during the ESI process. A natural concern is that the emitters may introduce enough heat, or introduce heat in an uncontrolled fashion, and promote the thermal degradation of these compounds under ESI conditions. Shown in Figure 11 is the analysis of the chemotherapy agent, taxol, a compound that degrades if exposed to too high a temperature. Using a vaporizer temperature of 150 °C, taxol had essentially no response in APCI mode (top). It was analyzed successfully by positive ESI-only mode or positive mixed mode (middle and bottom). Only minor peaks appeared in the mass spectra other than the protonated molecular ion, attesting to the lack of degradation products. The gentleness of the heating by the IR emitters is attributable to the vapor temperature sensor and feedback control which enable accurate, rapid and precise adjustment of the vapor temperature at the setpoint. Another example of the analysis of a thermally labile compound was presented previously in Figure 6, the spectrum of bovine insulin.

Higher HPLC flow rates: IR emitters and temperature feedback

Typical analytical HPLC flow rates are in the realm of 0.25–0.4 mL/min using 2.1 mm columns. However, high throughput analysis is generally performed on 4.6 mm ID columns, injecting several hundred samples per day into an LC/MS, with column flow rates between 1–2 mL/min. A dedicated ESI source normally has poor sensitivity at these higher flow rates and is operated with a postcolumn split, diverting the majority of the split flow to waste. This works but is inconvenient and needs additional parts in the system. Furthermore, operation in APCI mode becomes more attractive at higher flow rates as the analyte signal generally increases at higher flows.

As mentioned previously, the IR emitters emit enough power to vaporize HPLC effluents at up to 2 mL/min flow rate. The data in Figure 9 was obtained at 1 mL/min flow rate and illustrates that the feedback control is rapid enough to track a step change in temperature or mobile phase composition, coming to a steady state in a matter of 20–30 seconds. Data shown in Figures 12 and 13 were obtained at even higher flow rates, 1.5 mL/min.

LC Conditions

Column:	50 x 2.1 mm ZORBAX SB-C8, 5 µm (Agilent part no. 860975-906)
Mobile phase:	A = water with 0.05% formic acid B = acetonitrile
Gradient:	10% B at 0 min 95% B at 7 min Stop run at 8 min
Flow rate:	0.4 mL/min
Column temperature:	30 °C
Injection volume:	1 µL

MS Conditions

Source:	Multimode, APCI-only, ESI-only or mixed mode
Drying gas flow:	5 L/min
Nebulizer:	20 psig APCI, 60 psig ESI, 40 psig mixed-mode
Drying gas temp:	200 °C
Vaporizer temp:	150 °C
Capillary voltage:	2000 V
Corona current:	6 µA APCI, 2 µA mixed-mode
Peakwidth:	0.1 min
Time filter:	On
Scan:	100 –1000 *m/z*
Fragmentor:	150 V
Stepsize:	0.1 m/z

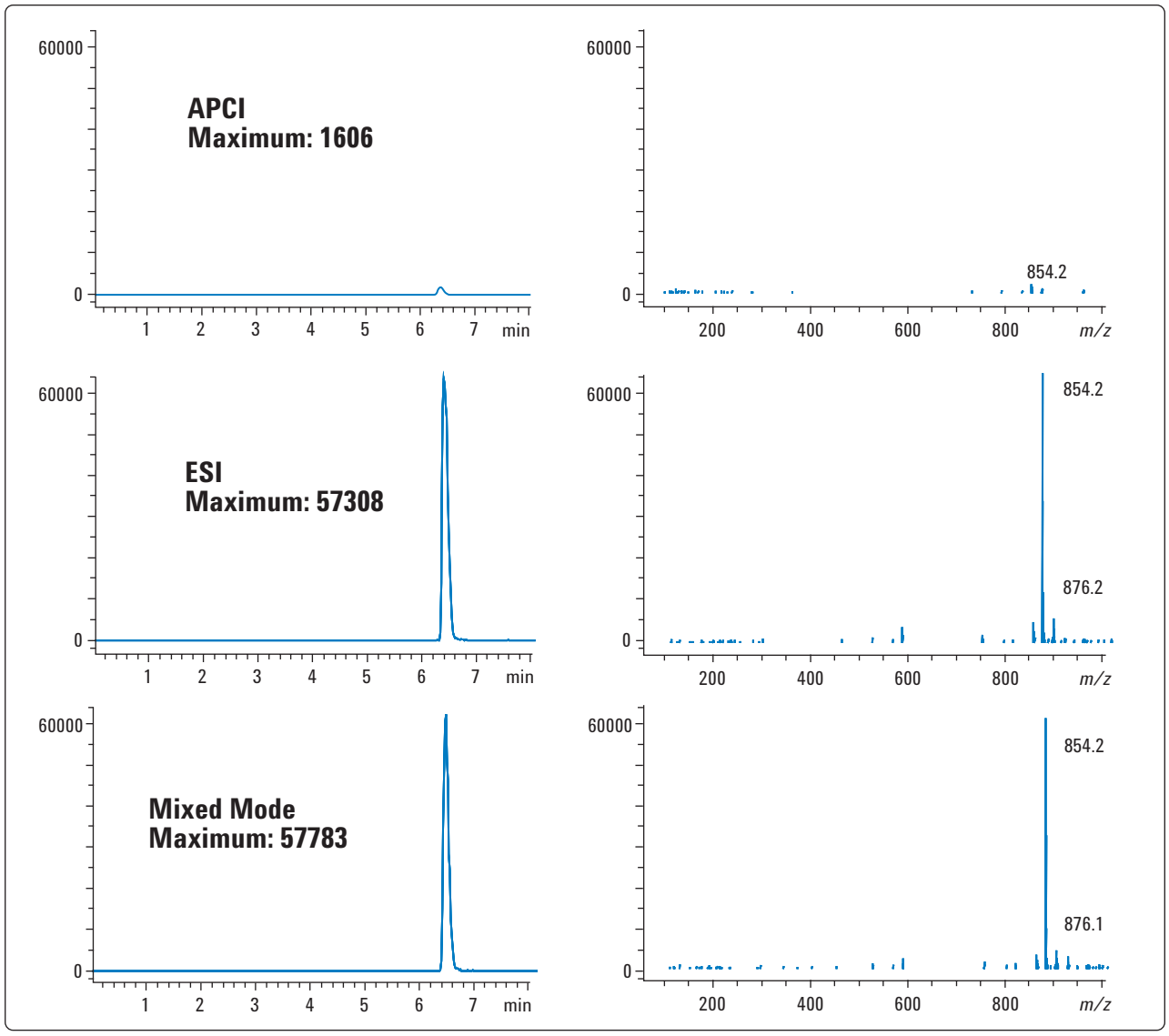

Figure 11. Extracted ion chromatograms of *m/z* 854.2 and spectra for the thermally labile compound taxol (1ng/µL) using the multi-mode source. (top) APCI-only mode, (middle) ESI-only mode, (bottom) mixed mode operation. Vaporizer setpoint was 150 °C. The response in ESI-only and mixed mode was quite similar, and the spectra show few mass peaks at *m/z* values less than the $[M+H]^+$ ion, signifying that there was little thermal degradation of taxol under these conditions.

Higher HPLC flow rates: Mixed mode operation yields better data for fast-eluting chromatographic peaks

It is possible to analyze samples either by mixed mode ESI and APCI or by alternating between the two ionization techniques on a scan-to-scan basis. When acquiring data, the instrument uses either one signal for mixed mode or two signals for alternating operation. There is a difference in the data quality between the two operational modes which becomes apparent when analyzing the narrower chromatographic peaks obtained during fast chromatography (Figure 12). Two compounds, taxol and progesterone, were analyzed using isocratic conditions in positive mixed mode (one signal, top trace) and in alternating ESI and APCI modes (two signals, the ESI signal is the middle trace). All other parameters: mass range, a/d sampling rate, etc. were held constant. (The signal from the UV detector is shown in the bottom trace for comparison.) The effect on the data is obvious, as about two times more scans across the peak were obtained when using mixed mode than when alternating modes. The reduction in chromatographic peak fidelity was caused by the fact that the instrument was monitoring two signals instead of one. Looking at it another way, in alternating mode operation, the instrument was not acquiring ESI data half of the time and was not acquiring APCI data during the other half of the time. But in mixed mode operation, the instrument was acquiring ESI data and APCI data all of the time. Note, the time it takes to switch between ESI and APCI is only a minor contributor to the differences observed. If the switching time were instantaneous, the instrument would still acquire twice as many scans across the peak in mixed mode operation. Therefore, mixed mode is in general the preferred mode of operation.

LC Conditions

Column:	15mm x 4.6 mm ZORBAX RRHT SB-C18, 1.8 µm (Agilent part no. 821975-902)
Mobile phase:	A = water with 0.2% acetic acid B = acetonitrile with 0.2% acetic acid
Gradient:	None (isocratic), 45% A, 55% B
Flow rate:	1.5 mL/min
Column temperature:	40 °C
Injection volume:	1 µL
Diode-array detector:	Signal 240, 10 nm; reference 340. 20 nm

MS Conditions

Source:	Multimode, positive mixed mode or alternating ESI and APCI
Drying gas flow:	7 L/min
Nebulizer:	60 psig
Drying gas temp:	200 °C
Vaporizer temp:	175 °C
Capillary voltage:	1500 V
Corona current:	2 µA
Peakwidth:	0.04 min
Time filter:	Off
Scan:	200–900 *m/z*
Fragmentor:	120 V
Stepsize:	0.1 *m/z*

Putting it all together: applications examples

High throughput analysis

High throughput analysis benefits from all the features of the multimode source: good ESI sensitivity, good APCI sensitivity, ability to handle higher HPLC flow rates, better chromatographic performance with mixed mode operation and higher HPLC flow rates, and better productivity in mixed mode operation. Faster injection-to-injection cycle time is obtained by combining these features with optimized HPLC techniques: short columns with 1.8 micron particle size, higher HPLC flow rates, minimized delay volume, overlapped injections, and alternating column regeneration.[6] A test set composed of pharmaceuticals and their intermediates was analyzed,

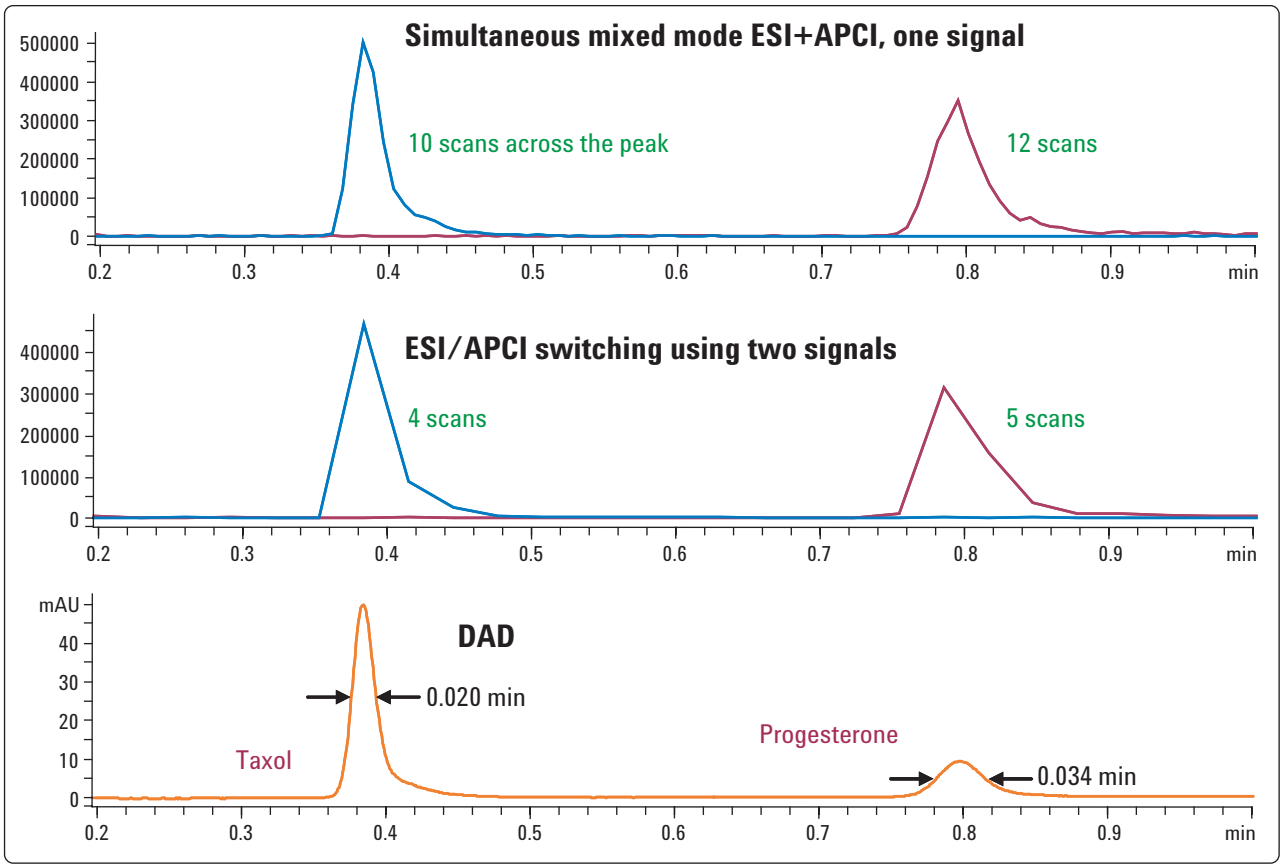

Figure 12. Simultaneous (mixed mode) operation vs. alternating mode in the analysis of taxol and progesterone. Using one signal for mixed mode operation (top) resulted in better chromatographic peak fidelity than using two signals for alternating mode operation (middle, only the ESI signal shown). The DAD signal is shown (bottom) for comparison. Even if the switching time were instantaneous, mixed mode operation would yield twice the number of scans across the peak because only one signal is being monitored, not two.

and one result from this set is shown in Figure 13. This represents a single injection of 100 ng progesterone analyzed in positive/negative mode and mixed mode operation. The top chromatogram is the positive mode TIC, second chromatogram is the negative mode TIC, the third chromatogram is the target mass trace, and the fourth chromatogram is the UV chromatogram. The spectrum at the apex of the target mass chromatogram peak is shown at the bottom. A gradient of 15–100% methanol in 0.75 minutes was used, thus reducing the injection-to-injection cycle time to about 1.75 minutes. As Figure 14 shows, an entire 96-wellplate can be analyzed in under three hours by using such a system. Of the compounds in the test set (Table 1), 89% were detected using a dedicated APCI source, and a different 89% were detected using a dedicated ESI source. However, all test compounds were detected using the multimode source in positive/negative mode, attesting to the greater utility of the multimode source.

LC Conditions

Columns:	Two alternating 15 x 4.6 mm ZORBAX RRHT SB-C18, 1.8 µm (Agilent part no. 821975-902)
Binary pump mobile phase:	A = water with 0.2% acetic acid B = methanol with 0.2% acetic acid
Gradient:	15% B at 0 min 100% B at 0.75 min 100% B at 1.00 min 15% B at 1.01 min Stop run at 1.50 min
Binary pump flow rate:	1.5 mL/min
Column temperature:	40 °C
Isocratic regeneration pump mobile phase:	85:15 water:methanol with 0.2% acetic acid
Isocratic pump flow rate:	1.5 mL/min
Injection volume:	1 µL, overlapped injection and minimized delay volume
Diode-array detector:	Signal 250 nm, 10 nm; reference off

MS Conditions

Source:	Multimode, mixed mode with positive/ negative switching
Drying gas flow:	5 L/min
Nebulizer:	40 psig
Drying gas temperature:	350 °C
Vaporizer temperature:	200 °C
Capillary voltage:	1500 V (positive and negative)
Corona current:	4 µA (positive and negative)
Peakwidth:	0.07 min
Time filter:	Off
Fast scan mode:	Enabled
Scan:	100–1150 *m/z*
Fragmentor:	120 V (positive and negative)
Stepsize:	0.3 *m/z*

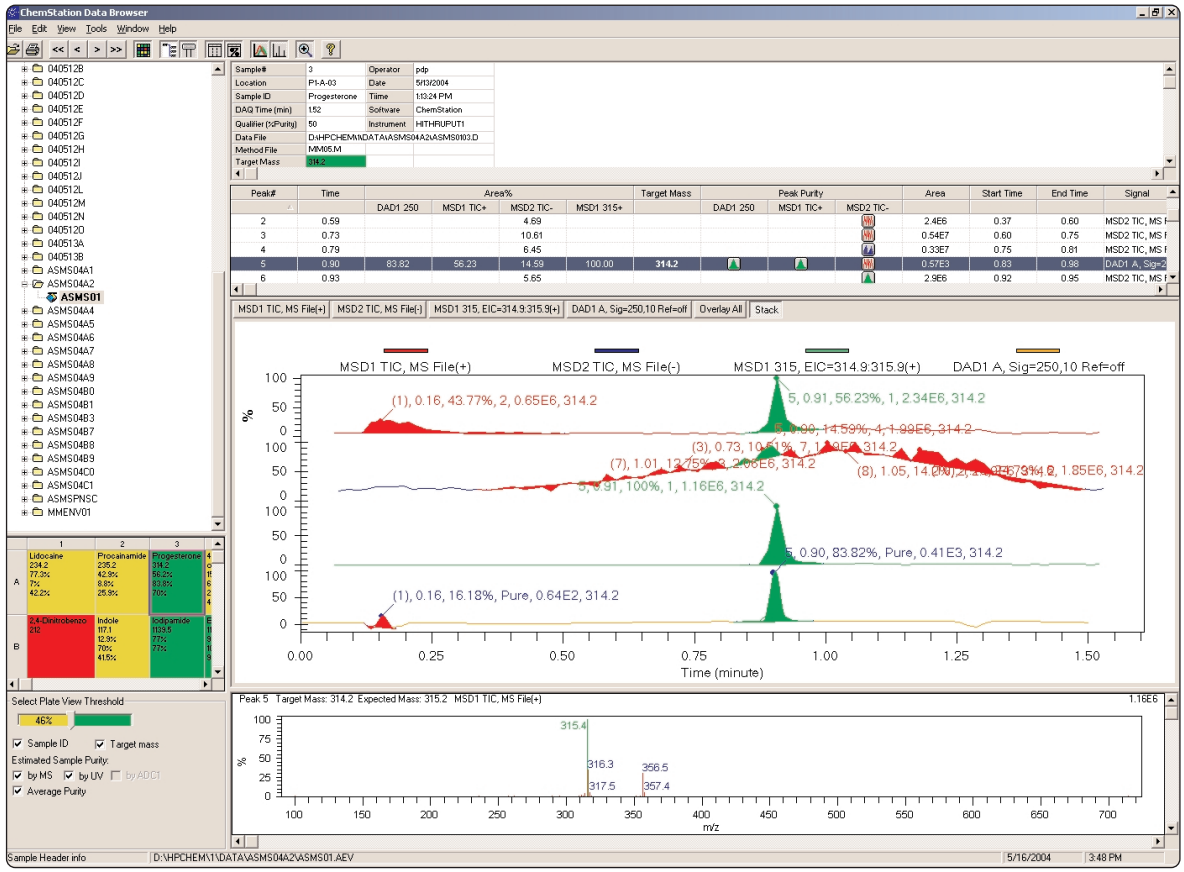
ChemStation Data Browser
MSD1 TIC, MS File(+)
MSD2 TIC, MS File(-)
MSD1 315, EIC=314.9:315.9(+)
DAD1 A, Sig=250,10 Ref=off
(1), 0.16, 43.77%, 2, 0.65E6, 314.2
5, 0.91, 56.23%, 1, 2.34E6, 314.2
5, 0.90, 83.82%, Pure, 0.41E3, 314.2
(1), 0.16, 16.18%, Pure, 0.64E2, 314.2
Time (minute)
m/z

Figure 13. High throughput analysis of progesterone (100 ng on column). (top trace) positive mode TIC, (second from top trace) negative mode TIC, (third from top trace) positive mode target ion *m/z* 315.2, (fourth from top trace) DAD signal, (bottom) spectrum from peak apex of target mass trace.

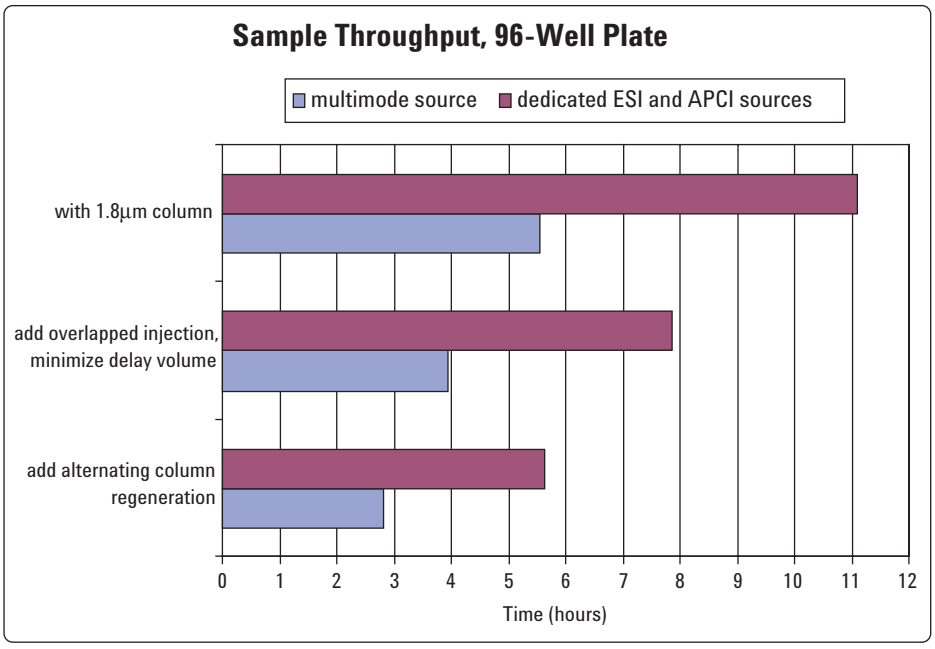

Figure 14. Improvement in sample throughput by combining analysis techniques and hardware. An entire 96-well plate can be analyzed in under three hours by combining all improvements.

Table 1. Summary of responses for pharmaceutical compounds and intermediates analyzed using various ion sources: (left two columns) analysis using a dedicated APCI source, (middle two columns) using a dedicated ESI source, (right two columns) using a multimode source in mixed mode. The multimode source detected all compounds in the test suite, whereas the dedicated sources did not.

	APCI Source		ESI Source		Multimode Source	
Compound	Positive	Negative	Positive	Negative	Positive	Negative
Acetazolamide	–	D	–	D	–	D
Butyl 4-aminobenzoate	D	–	D	–	D	–
Cortisone	D	D	D	–	D	D
Gemfibrozil	–	D	–	D	–	D
Hexahydro-...-dione	D	–	D	–	D	–
Hydroflumethiazide	–	D	–	D	–	D
Indole	D	–	–	–	D	–
Iodipamide	–	–	–	D	D	D
Labetalol	D	D	D	D	D	D
Lidocaine	D	–	D	–	D	–
Morin	D	D	D	D	D	D
Paclitaxel	–	–	D	–	D	–
Phenylbutazone	D	D	D	D	D	D
Procainamide	D	–	D	–	D	–
Progesterone	D	–	D	–	D	–
Sulfamethoxazole	D	D	D	D	D	D
Tolazamide	D	D	D	D	D	D
Uracil	–	D	–	–	–	D
Detected by polarity	**67%**	**56%**	**67%**	**50%**	**78%**	**61%**
Detected by source	**89%**		**89%**		**100%**	

D = compound detected

***Hexahydro-...dione = Hexahydro-2,6-bis(2,2,6,6-tetramethyl-4-piperidinyl)-1H,4H,5H,8H-2,3a,4a,6,7a,8a-hexaazacyclopenta[def]fluorene-4,8-dione**

Steroids analysis

Steroids are known to be difficult to analyze; some respond well in ESI, and others respond better in APCI mode. Investigators sometimes derivatize them in an effort to improve their detection limits.[7] Several steroids with different functional groups (acid, ketone, fluoride, hydroxyl) were analyzed successfully, showing a strong response under positive/negative mixed mode conditions (Figure 15).

Environmental compound screening

Figure 16 shows the analysis of several classes of compounds of interest in environmental compound screening.[8] Representative pesticides, herbicides and insecticides (bipyridyliums, triazines, phenylureas, carbamates, phenoxyacetic acids, phenols) were analyzed at the 5 ng level in positive/negative mixed mode. All compounds gave a response that was visible in the TIC. This is especially noteworthy as the broad range of compound types, chromatography, and coelution dictate that the pH and vaporization conditions were not optimal for every analyte in the sample.

LC Conditions

Column:	30 x 2.1 mm ZORBAX SB-C18, 3.5 µm (Agilent part no. 873700-902)
Mobile phase:	A = water with 0.2% acetic acid B = methanol with 0.2% acetic acid
Gradient:	10% B at 0 min 10% B at 0.5 min 100% B at 9 min Stop run at 12 min
Flow rate:	0.4 mL/min
Column temperature:	40 °C
Injection volume:	1 µL

MS Conditions

Source:	Positive/negative switching multimode, mixed mode
Drying gas flow:	5 L/min
Nebulizer:	40 psig
Drying gas temp:	350 °C
Vaporizer temp:	200 °C
Capillary voltage:	1500 V (positive and negative)
Corona current:	4 µA (positive and negative)
Peakwidth:	0.12 min
Time filter:	On
Scan:	165–600 *m/z*
Fragmentor:	130 V (positive and negative)
Stepsize:	0.1 *m/z*

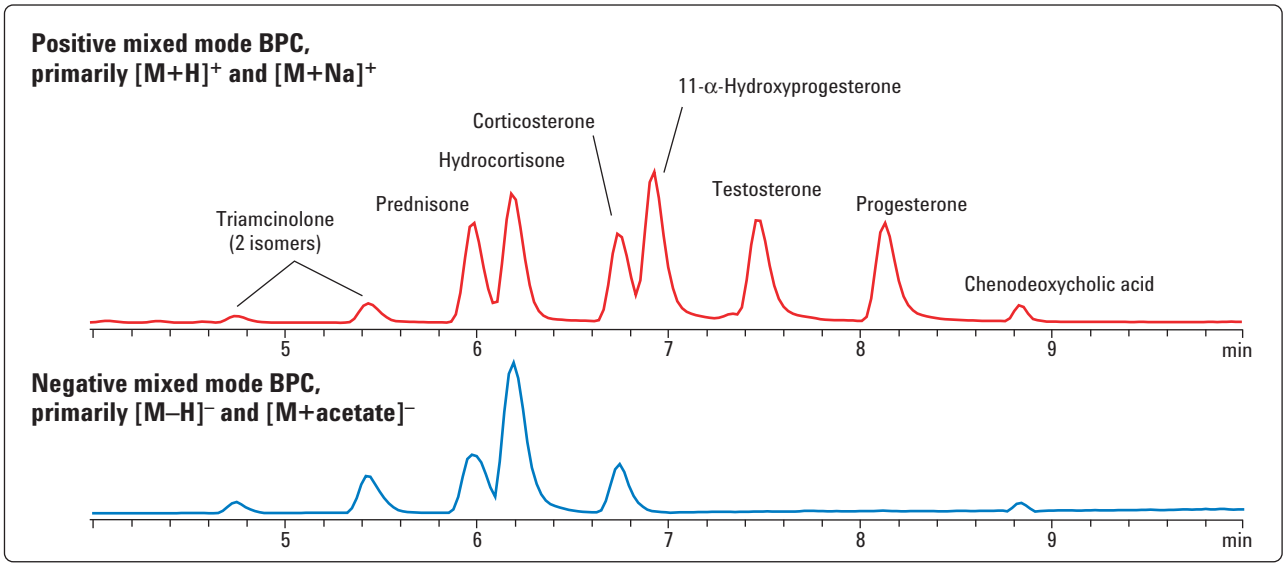

Figure 15. Analysis of underivatized steroids (100 ng per component) using the multimode source, mixed mode with positive/negative polarity switching. A wide range of functional groups was represented.

LC Conditions

Sample:	1 ng per component, dissolved in 80:20 water:methanol with 1% acetic acid
Column:	150 x 2.1 mm ZORBAX Eclipse XDB-C18, 3.5 µm (Agilent part no. 930990-902)
Mobile phase:	A = water with 1 mM ammonium acetate B = methanol with 1 mM ammonium acetate
Gradient:	3% B, 0.3 mL/min at 0 min 90% B, 0.3 mL/min at 45 min 3% B, 0.3 mL/min at 45.01 min 3% B, 0.5 mL/min at 45.02 min 3% B, 0.5 mL/min at 50 min 3% B, 0.3 mL/min at 50.01 min Stop run at 52 min
Flow rate:	0.3 mL/min
Column temperature:	60 °C
Injection volume:	5 µL

MS Conditions

Source:	Multimode, mixed mode with positive/negative switching
Drying gas flow:	6 L/min
Nebulizer:	40 psig
Drying gas temp:	300 °C
Vaporizer temp:	150 °C
Capillary voltage:	1000 V (positive and negative)
Corona current:	2 µA (positive and negative)
Peakwidth:	0.3 min
Time filter:	On
Scan:	130–330 *m/z*
Fragmentor:	130 V (positive and negative)
Stepsize:	0.1 *m/z*

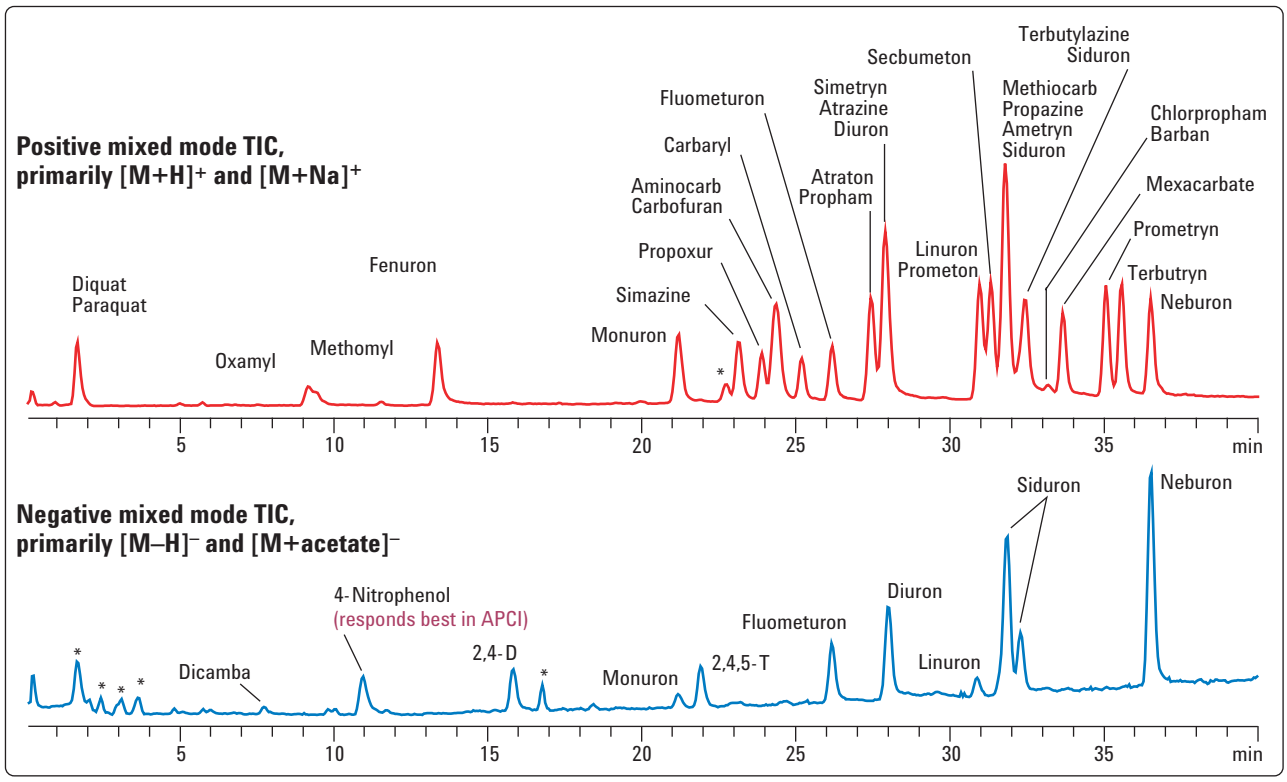

Figure 16. Analysis of compounds of environmental interest (5 ng per component) using the multimode source, mixed mode with positive/negative polarity switching. A wide range of compound classes was represented (bipyridyliums, triazines, phenylureas, carbamates, phenoxyacetic acids, phenols). *Denotes impurity

Conclusions

The Agilent multimode source contains several novel design elements which are patented or in the process of being patented.[9] These elements and the method of their use allow the multimode source to perform ESI, APCI or simultaneous ESI and APCI at higher HPLC flow rates with comparable sensitivity to dedicated ion sources. Switching between modes is as simple as clicking the mouse or may be programmed automatically. Mixed mode operation permits higher quality data to be obtained on narrow chromatographic peaks, such as those found in high throughput analysis. Nitrogen gas consumption is reduced significantly using this source rather than a dedicated ESI source. The ultimate benefits of the source are increased sample throughput, better scheduling of samples, better use of instrument time, lower operating costs, and less time spent reanalyzing samples.

References

1. Cody, R.B. Electrospray Ionization Mass Spectrometry: History, Theory, and Instrumentation. In *Practical Spectroscopy Series, Volume 32, Applied Electrospray Mass Spectrometry,* Pramanik, B.N.; Ganguly, A.K.; Gross, M.L., Eds.; Marcel Dekker, Inc.: New York, NY, 2002; chapter 1.
2. Gaskell, S.J. Electrospray: Principles and Practice. *J. Mass Spectrom.* **1997,** 32, 677–688.
3. Niessen, W.M.A.; Tinke, A.P. Liquid chromatography-mass spectrometry general principles and instrumentation. *J. Chrom. A* **1995,** 703, 37–57.
4. Siegel, M.M.; Tabei, K; Lambert, F.; Candela, L.; Zoltan, B. J. *Am. Soc. Mass Spectrom.* **1998,** 9, 1196–1203.
5. Gallagher, R.T.; Balogh, M.P.; Davey, P.; Jackson, M.R.; Sinclair, I.; Southern, L.J. Combined electrospray ionization-atmospheric pressure chemical ionization source for use in high-throughput LC-MS applications. *Anal. Chem.* **2003,** 75, 973–977.
6. Huber, U. High throughput HPLC—Alternating column regeneration with the Agilent 1100 Series valve solutions. *Agilent Technologies application note* **2002,** publication number 5988-7831EN.
7. Higashi, T.; Shimada, K. Derivatization of neutral steroids to enhance their detection characteristics in liquid chromatography–mass spectrometry. *Anal. Bioanal. Chem.* **2004,** 378, 875–882.
8. Thurman, E.M.; Ferrer, I.; Barcelo, D. Choosing between Atmospheric Pressure Chemical Ionization and Electrospray Ionization Interfaces for the HPLC/MS Analysis of Pesticides. *Anal. Chem.* **2001,** 73, 5441–5449.
9. US patent 6,646,257; others pending.

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